REMARKS

1. Objections to the Specification

The Examiner objected to the Specification due to blanks relating to the listed priority documents. Applicants have updated the specification as suggested by the Examiner.

The Examiner also objected to the Specification due to instances in the specification where sequences are not labeled with a corresponding SEQ ID NO. Applicants have amended the specification and the Sequence Listing to correct this informality. Applicants submit that the Sequence Listing and these amendments merely embody the correction of formal matters in the specification and as such include no new matter. Applicants enclose herewith a substitute paper copy and computer readable form of the Sequence Listing. The computer readable form and paper copy were previously submitted in U.S. Application Serial No. 08/460,428, filed June 2, 1995, of which the present application is a divisional. Applicants request that the USPTO change the application data on page 1 of the Sequence Listing to reference this divisional, if necessary. Applicants assert pursuant to 37 CFR § 1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:17 submitted herewith are identical.

2. Rejection of Claims 43-54 under 35 U.S.C. 112, first paragraph.

Claims 43-54 were rejected under 35 U.S.C. 112, first paragraph. The Examiner argues that the specification does not reasonably provide enablement for an antibody which selectively binds a filariid p22U protein, i.e. the Examiner argues that the specification is silent concerning the reactivity/crossreactivity of the antibodies in the immune serum.

In order to expedite allowance of claims, Applicants have, without prejudice or disclaimer of the subject matter thereof, amended Claims 44 and 52 to recite monoclonal antibodies that bind to a protein comprising amino acid sequence SEQ ID NO:4. Applicants have also added Claim 55, which recites an isolated antibody raised using an isolated *D. immitis* p22U protein. Support for these amendments may be found in the specification at page 45, lines 11 through page 46, line 20. These amendments are believed to obviate the Examiner's concerns. However, with regard to the Examiner's contention that the specification is silent concerning the binding of any other proteins to the immune serum, Applicants respectfully submit that the Examiner is incorrect in this statement.

Applicants refer the Examiner to page 78, lines 2-9, which indicates that immune serum selectively binds to recombinant *D. immitis* p22U protein, and does not bind to lysates of cells transformed with only the pTrcHisB plasmid (empty vector).

In view of the foregoing, Applicants respectfully request withdrawal of the Examiner's rejection of Claims 43-54 under 35 U.S.C. 112, first paragraph.

3. Rejection of Claims 46 and 52-54 under 35 U.S.C. 112, first paragraph.

Claims 46 and 52-54 were rejected under 35 U.S.C. 112, first paragraph. The Examiner argued that the specification does not reasonably provide enablement for a composition or method of protecting the recipient animal from infection.

Initially, Applicants note that Claims 46 and 54 have been canceled. In order to expedite allowance of claims, Applicants have, without prejudice or disclaimer of the subject matter thereof, amended Claims 52 and 53 to recite a composition comprising an excipient and an isolated antibody that binds to a protein comprising amino acid sequence SEQ ID NO:4.

In view of the foregoing, Applicants respectfully request withdrawal of the Examiner's rejection of Claims 46 and 52-54 under 35 U.S.C. 112, first paragraph.

4. Rejection of Claims 43-48 and 50-54 under 35 U.S.C. 112, first paragraph.

Claims 43-48 and 50-54 were rejected under 35 U.S.C. 112, first paragraph. The Examiner argued that the specification does not reasonably provide enablement for antibodies which bind to any and all other filariid p22U proteins or methods for cross protection against any and all other filariids.

Initially, Applicants note that Claims 44, 46-48, 51 and 54 have been canceled. In order to expedite allowance of claims, Applicants have, without prejudice or disclaimer of the subject matter thereof, amended Claims 43, 45, 50, and 52-53 to recite an isolated antibody that binds to a protein comprising amino acid sequence SEQ ID NO:4 or a composition of such an antibody.

In view of the foregoing, Applicants respectfully request withdrawal of the Examiner's rejection of Claims 43-48 and 50-54 under 35 U.S.C. 112, first paragraph.

5. Rejection of Claims 43-53 under 35 U.S.C. 102(b).

Claims 43-53 were rejected under 35 U.S.C. 102(b) as anticipated by Tulloch et al. The Examiner noted that Tulloch et al. teach the production of antibodies by immunizing dogs with larval stage *D. immitis* and collection of whole sera from said dogs and argued that the whole sera of Tulloch et al. would inherently comprise the antibodies of the claimed invention.

Applicants note that the claims have been amended to recite an isolated monoclonal antibody that binds to a protein comprising amino acid sequence SEQ ID NO:4 or a composition of such an antibody. Accordingly, Applicants respectfully argue that the claims as amended are not anticipated by Tulloch et al. under 35 U.S.C. 102(b). Claims are anticipated if, and only if, each and every element as set forth in the claims is found in a single prior art reference. Verdegaal Bros. V. Union Oil Co. of California, 2 USPQ2d 1051 (Fed. Cir. 1989). Furthermore, "[t]he identical invention must be shown in as complete detail as is contained in the . . . claim." Richardson v. Suzuki Motor Co., 9 USPQ2d 1913 (Fed. Cir 1989). Applicants submit that significant differences exist between the claimed invention and disclosure of Tulloch et al. in that the instant application describes and claims monoclonal antibodies which bind to an amino acid sequence that was first isolated and sequenced by the present inventors. While Tulloch et al. do teach injection of dogs with whole *D. immitis* larvae and collection of whole sera from such dogs, such a general disclosure does not rise to the level of anticipating claims drawn to an isolated monoclonal antibody against a specific protein component of *D. immitis* larvae.

With respect to Claim 45, Applicants respectfully note that Claim 45 recites an isolated antibody of Claim 43, wherein the protein bound by the antibody of Claim 43 selectively binds to immune serum. Support for this claim may be found in the specification in Example 1 (native p22U protein) and in Example 7 (recombinant p22U protein).

Applicants further note that added Claims 55-59 recite an isolated antibody raised using an isolated *D. immitis* p22U protein. Such an antibody is not anticipated by Tulloch et al., which does not teach or contemplate raising antibodies using specific, isolated proteins.

In view of the foregoing, Applicants respectfully request withdrawal of the Examiner's rejection of Claims 43-53 under 35 U.S.C. 102(b).

6. Amendment of Inventorship under 37 C.F.R. 1.48(b)

Applicants have reviewed the pending claims of the present application and determined that prosecution of the present application resulted in the amendment or cancellation of claims which necessitates a change of inventorship. Therefore, Applicants are correcting inventorship by submission of a petition as required by 37 C.F.R. § 1.48(b) and the fee set forth in 37 C.F.R. §1.17(i). By way of this amendment of inventorship, originally named inventor Cynthia A. Tripp has been removed as inventor. The remaining inventors are Glenn R. Frank and Robert B. Grieve.

In view of the foregoing, Applicants respectfully assert that all pending claims are in a condition for allowance. In the event that the Examiner has any questions regarding Applicants; position, the Examiner is invited to contact the below-named representative.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

On page 1, the paragraph spanning lines 3-14 was amended as follows:

--The present application is a divisional application of U.S. Application Serial No. 08/460,428, filed June 2, 1995, entitled "PARASITIC HELMINTH P22U PROTEINS, now U.S. Patent No. 5,912,337, issued on June 15, 1999, which is a continuation-in-part of U.S. Patent Application Serial No. 08/003,257, filed January 12, 1993, entitled "Reagents and Methods for Identification of Vaccines"; of U.S. Patent Application Serial No. 08/003,389, filed January 12, 1993, entitled "Immunogenic Larval Proteins"; and of U.S. Patent Application Serial No. 07/654,226, filed February 12, 1991, entitled "Reagents and Methods for Identification of Vaccines". Serial Nos. 08/003,257 and 08/003,389 are also continuation-in-parts of Serial No. 07/654,226. Each of these applications is incorporated by reference herein in their entireties --.

The paragraph spanning page 24, line 23 through page 25, line 19 was amended as follows:

--The protein encoded by *D. immitis* p4 is further characterized by having an LDL receptor-related protein (LDLr) class A cysteine-rich motif of about 9 amino acids that is also found in several other proteins, including mammalian low density lipoprotein (LDL) receptors, LDL receptor-related proteins, human and mouse alpha-2-macroglobulin receptors and rat renal GP 330 glycoprotein. Each of these proteins, including *D. immitis* P4, share the sequence DDCGDGSDE (i.e., Aspartic Acid -- Aspartic Acid -- Cysteine -- Glycine -- Aspartic Acid -- Glycine -- Serine -- Aspartic Acid -- Glutamic Acid), denoted herein as SEQ ID NO:5. A conserved stretch of eight of the nine amino acids is also found in the free-living (i.e., non-parasitic) nematode *Caenorhabditis elegans* LDL receptor-related protein and *C. elegans* basement membrane proteoglycan. This LDLr class A, cysteine-rich motif is likely to be conserved in proteins encoded by p4-related sequences of other helminths (i.e., nucleic acid sequences that hybridize under stringent conditions with *D. immitis* p4). As such, p4-related nucleic acid sequences may be identified using oligonucleotide probes that encode such LDLr class A motifs. Furthermore, the LDLr class A motif in P4-related proteins

represents a target for development of therapeutic compositions to protect animals from parasitic helminth infection, as discussed below--.

On page 68, the paragraph spanning lines 2-24 was amended as follows:

--The chromatogram depicting the tryptic fragments of P22U is shown in FIG. 2. The fragments indicated by asterisks were submitted for sequencing. All sequencing was conducted at Macromolecular Resources, Department of Biochemistry, Colorado State University, Fort Collins, Colorado. The peptides were concentrated to 50 μl or less using a Speedvac® and frozen at about -20°C until sequencing. N-terminal sequencing was conducted in an ABI Model 473A Protein/ Peptide Sequencer System (Applied Biosystems, Inc., Foster City, California) using pulsed liquid chemistry and on line microgradient PTH amino acid analysis (see, for example, Hewick et. al., 1981, *J. Biol. Chem.* 256, p. 7990-7997; Geisow and Aitken, 1989, in Findlay, J.B.C. and M.J. Geisow (ed.). *Protein Sequencing: A Practical Approach*, p. 85-98). The most likely sequence of the tryptic fragment eluting at 44 minutes (referred to as the 44 min tryptic fragment), using one-letter amino acid code, was MAQDAFPNACAQGEPK (SEQ ID NO:6). The most likely sequence of the tryptic fragment eluting at 58 minutes (referred to as the 58 min tryptic fragment) was AIAPCQLTAVQSVLPCADQCQK (SEQ ID NO:7). The most likely sequence of the tryptic fragment eluting at 60 minutes (referred to as the 60 min tryptic fragment) was LGSCSPDCGLDLPSDNVMVQDV (SEQ ID NO:8)--.

On page 71, the paragraph spanning lines 10-22 was amended as follows:

--A homology search of the non-redundant protein sequence database was performed through the National Center for Biotechnology Information using the BLAST network. This database includes SwissProt + PIR + SPUpdate + GenPept + GPUpdate. The search was performed using SEQ ID NO:2 and showed the only significant homology shared between SEQ ID NO:2 and known sequences to be a contiguous stretch of 9 amino acids, namely DDCGDGSDE (SEQ ID NO:5), that was also found in human LDL-receptor related protein, human and mouse alpha-2-macroglobulin receptors and rat renal GP 330 glycoprotein. A conserved stretch of eight of the nine amino acids

is also found in *Caenorhabditis elegans* LDL receptor-related protein and *C. elegans* basement membrane proteoglycan.--

The paragraph spanning page 71, line 26 through page 72, line 18 was amended as follows:

--Recombinant molecule pET19b-$p4_{635}$, containing *D. immitis* p4 nucleotides from about 1 through about 635 operatively linked to bacteriophage T7*lac* transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 10 histidines was produced in the following manner. An about 635-nucleotide DNA fragment containing nucleotides spanning from about 1 through about 635 of SEQ ID NO:1, called $p4_{635}$, was PCR amplified from a clone containing *D. immitis* p4 using the primers 5' CGGGATCCCGAGTTAAATAGTCG 3' (denoted SEQ ID NO:9 or 394-5'; *Bam*HI site underlined) and 5' TGCAGGATCCTGCACCG 3' (denoted SEQ ID NO:10 or 394-3'; *Bam*HI site underlined).. The PCR product was digested with *Bam*HI restriction endonuclease, gel purified and subcloned into expression vector pET19b (available from Novagen Inc., Madison, WI) that had been cleaved with *Bam*HI. The resulting recombinant molecule pET19b-$p4_{635}$ was transformed into *E. coli* BL21(DE3)pLysS to form recombinant cell *E. coli*:pET19b-$p4_{635}$. *E. coli* BL21(DE3)pLysS includes a bacteriophage T7 RNA polymerase gene under the control of *lac* transcription control sequences.--

On page 74, the paragraph spanning lines 2-27 was amended as follows:

--A segment of DNA for use in the identification of a nucleic acid sequence capable of encoding at least a portion of P22U was produced by PCR amplification using standard techniques, such as those described in Sambrook et al., *ibid.* Briefly, first strand cDNA was synthesized from adult female poly A+ RNA using Murine Leukemia Virus reverse transcriptase (available from Stratagene) and Stratagene's linker-primer from their ZAP-cDNA Synthesis Kit, namely 5' GAGAGAGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTTTTT- TTTTTTTT 3' (SEQ ID NO:11). A pool of two sets of degenerate primers was produced based on the partial amino acid sequence of the 60 min tryptic fragment described in Example 3. One degenerate set of primers, denoted GRF 11, includes the following sequences: 5'TGYTCNCCNGAYTGYGG 3' (SEQ ID NO:12), wherein Y can be either C or T, and N can be either A, G, C or T. The second set of

primers, denoted GRF 12, includes the following sequences: 5'TGYAGTCCNGAYTGYGG 3' (SEQ ID NO:13). PCR amplification using the pool of degenerate primers in combination with Stratagene's linker-primer as the antisense primer was used to amplify the DNA segment. Verification that the appropriate segment had been amplified was accomplished by Southern blot analysis using a degenerate probe based on a more C-terminal amino acid sequence of the 60 min tryptic fragment, namely GRF 3 which includes the following sequences: 5' TGNACCATNACRTTRTC 3' (SEQ ID NO:14), wherein R can be either A or G.--

On page 75, the paragraph spanning lines 9-20 was amended as follows:

--The adult female cDNA library was screened with an antisense probe, using stringent (i.e., standard) hybridization conditions as described in Sambrook et al., *ibid.*. The antisense probe, denoted GRF14, was based on the DNA sequence derived from the amplified segment and has the sequence 5' CTGTTTGAACCATAACATTATCAGATGG 3' (SEQ ID NO:15). Plaques which hybridized to the probe were rescreened, plaque purified and clones containing *D. immitis* nucleic acid sequence p22U (i.e., clones that hybridized with the antisense probe and having the apparent nucleic acid sequence designated in SEQ ID NO:3) were submitted to nucleic acid sequencing as described in Example 4.--

The paragraph spanning page 77, line 1 through page 78, line 1 was amended as follows:

--Recombinant molecule pHis-p22U_{608}, containing *D. immitis* p22U nucleotides from about 41 through about 649 operatively linked to *trc* transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidines was produced in the following manner. An about 608-nucleotide DNA fragment containing nucleotides spanning from about 41 through about 649 of SEQ ID NO:3, called p22U_{608}, was PCR amplified from a clone containing *D. immitis* p22U using the primers 5' GTTGCAAT- ATGGGATCCAATGAGCC 3' (denoted SEQ ID NO:16 or 22USEN; *Bam*HI site underlined) and 5' CGCTAGTGCAGGATCCTCAATACTC 3' (denoted SEQ ID NO:17 or 22UANT; *Bam*HI site underlined). The PCR product was digested with *Bam*HI restriction endonuclease, gel purified and subcloned into expression vector pTrcHisB (available from Invitrogen) that had been cleaved with *Bam*HI. The resulting recombinant molecule

pHis-p22U$_{608}$ was transformed into *E. coli* to form recombinant cell *E. coli*:pHis-p22U$_{608}$. The recombinant cell was cultured in shake flasks containing an enriched bacterial growth medium containing 0.1 mg/ml ampicillin at about 37°C. When the cells reached an OD$_{600}$ of about 0.3, expression of *D. immitis* p22U$_{608}$ was induced by addition of about 1 mM IPTG. Protein production was monitored by SDS PAGE of recombinant cell lysates, followed by Coomassie blue staining, using standard techniques. Recombinant cell *E. coli*:pHis-p22U$_{608}$ produced a protein, denoted herein as PHIS-P22U$_{608}$, that migrated with an apparent molecular weight of about 27 kD. Such a protein was not produced by cells transformed with the pTrcHisB plasmid lacking a *D. immitis* DNA insert.--

IN THE CLAIMS:

Claims 44, 46-49, 51 and 54 were canceled without prejudice or disclaimer of the subject matter therein.

Claims 43, 45, 50, 52 and 53 were amended as follows.

43. (Once Amended) An isolated monoclonal antibody that selectively binds to a [filariid p22U] protein comprising amino acid sequence SEQ ID NO:4.

45. (Once Amended) The antibody of Claim 43, wherein said [filariid p22U] protein selectively binds to immune serum that inhibits [filariid] *D. immitis* development.

50. (Once Amended) The antibody of Claim 43, wherein said antibody selectively binds to a [p22U] protein encoded by a nucleic acid sequence [selected from the group consisting of] SEQ ID NO:3[, a nucleic acid sequence containing at least a portion of SEQ ID NO:3 and a fragment of SEQ ID NO:3].

52. (Once Amended) A [therapeutic] composition [that protects an animal from filariid infection, said composition] comprising an excipient and an isolated monoclonal

antibody that selectively binds to a protein comprising amino acid sequence SEQ ID NO:4[of Claim 43].

53. (Once Amended) The [therapeutic] composition of Claim 52, wherein said composition further comprises at least one component selected from the group consisting of [an excipient,] an adjuvant and a carrier.

Claims 55-59 were added as follows:

55. (Added) An isolated antibody raised using an isolated *D. immitis* p22U protein.

56. (Added) The antibody of Claim 55, wherein said antibody is a monoclonal antibody.

57. (Added) The antibody of Claim 55, wherein said antibody is a polyclonal antibody.

58. (Added) The antibody of Claim 55, wherein said protein is a recombinant protein.

59. (Added) The antibody of Claim 55, wherein said *D. immitis* p22U protein comprises amino acid sequence SEQ ID NO:4.